U. S. NAVAL SUBMARINE MEDICAL CENTER

Submarine Base, Groton, Conn.

REPORT NUMBER 604

EFFECTS OF CHRONIC HYPERCAPNIA ON BLOOD VOLUME, PLASMA VOLUME AND RED CELL VOLUME IN RELATION TO THE SUBMARINE ENVIRONMENT

by

George Thomas Baker III and Karl Ernst Schaefer

Bureau of Medicine and Surgery, Navy Department
Research Work Unit MF12.524.006-9028.05

Released by:

J. E. STARK, CAPT MC USN
COMMANDING OFFICER
Naval Submarine Medical Center

24 November 1969

EFFECTS OF CHRONIC HYPERCAPNIA ON BLOOD VOLUME, PLASMA VOLUME AND RED CELL VOLUME IN RELATION TO THE SUBMARINE ENVIRONMENT

by

George T. Baker, III
and
Karl E. Schaefer, M.D.

SUBMARINE MEDICAL RESEARCH LABORATORY
U.S. NAVAL SUBMARINE MEDICAL CENTER REPORT NO. 604

Bureau of Medicine and Surgery, Navy Department
Research Work Unit MF12.524.006-9028.05

Transmitted by:

Karl E. Schaefer, M.D.
Head, Physiology Branch

Reviewed and Approved by:

Charles F. Gell, M.D., D.Sc.(Med.)
Scientific Director
SubMedResLab

Reviewed and Approved by:

J. D. Bloom, CDR MC USN
Director
SubMedResLab

Approved and Released by:

J. E. Stark
Captain, MC, U. S. Navy
Commanding Officer, SubMedCen

SUMMARY PAGE

THE PROBLEM

To determine the effects of chronic hypercapnia on red cell and plasma volume, for which there is no apparent record of previous publications on the conduct of similar research.

FINDINGS

Red cell volume and plasma volume were measured simultaneously in guinea pigs exposed to 15% CO_2 using chromium^{-51} tagged red cells and iodinated human albumin (I^{125}). Red cell volume was found to increase during the uncompensated phase and to a greater extent during the compensated phase of respiratory acidosis while the plasma volume did not change significantly.

APPLICATION

These findings are of importance to environmental scientists and submarine medical officers who deal with CO_2 toxicity problems in closed spaces. Submarines are not as a rule exposed to the high concentrations of CO_2 as that used as a stressor mechanism in these experiments. There are however possibilities of CO_2 absorber temporary breakdowns in which the phenomena discussed in this report may be pertinent to the medical officer.

ADMINISTRATIVE INFORMATION

This investigation was conducted as a part of Bureau of Medicine and Surgery Work Unit MF12.524.006-9028 -- Time-Concentration Exposure Limits to Carbon Dioxide. The present report is No. 5 on this Work Unit. The manuscript was approved for publication on 24 November 1969 and designated as SubMedRes Lab Report No. 604.

PUBLISHED BY THE NAVAL SUBMARINE MEDICAL CENTER

ABSTRACT

Blood volume in guinea pigs, estimated on the basis of simultaneous measurement of red cell volume using chromium-51 tagged red cells and plasma volume with iodinated human albumin (I-125), was found to be more accurate than blood volume data calculated from separate determinations of red cell volume (chromium-51) and plasma volume (I-131). In chronic hypercapnia total blood and cell volume was found to increase during the uncompensated phase and increase to a greater extent during the compensated phase, while the plasma volume did not change significantly. The first rise was attributed to the release of blood stores from the spleen and liver while the later rise was attributed to an increased rate of erythropoiesis.

EFFECTS OF CHRONIC HYPERCAPNIA ON BLOOD VOLUME, PLASMA VOLUME AND RED CELL VOLUME IN RELATION TO THE SUBMARINE ENVIRONMENT

INTRODUCTION

It is considered a basic premise that long-term exposure to submersion in a submarine will result in chronically elevated CO_2 exposure. The concentrations of CO_2 used in these hypercapnia experiments are far greater than those normally found in a submarine, but were useful in delineating certain changes due to possible maximum exposure. The effects of chronic hypercapnia on circulating blood volume and actual blood volume in organs and tissues have not been reported, to our knowledge. The longest exposure to CO_2 in which measurements of circulating blood volume were published, so far, was carried out by Dagher, Pannossian and Fuleihan (1968) who failed to demonstrate changes in anesthetized dogs exposed to 5% CO_2 for four hours. However, acute effects of carbon dioxide on vascular resistance* and blood flow are well documented (Richardson, Wasserman and Patterson, 1960; Cooper, 1962; and Bygdeman, 1963).

There is general agreement that the effects of hypercapnia on most vasculature beds is reflex in nature, that is eliciting (1) an arterial and venous constrictive response mediated through the central nervous system, (2) through epinephrine discharge of the adrenals, and (3) a local vasodilatory response due to elevated arterial P_{CO_2}. The conditions under which one response predominates over the other have not been definitely established. However, it appears that in most tissues it is dependent upon the level of P_{CO_2} and the duration of the induced hypercapnia. The higher the P_{CO_2} and the longer the duration, the greater the vasodilatory response. Also, the type of response is apparently determined to some extent by the size of the vascular bed in a particular tissue. The larger the vascular bed, presumably the greater the blood volume, with the consequent exposure to higher concentrations of circulatory catecholamines which elicit a vasoconstrictive response.

To make any direct correlation between the blood content of a given tissue and blood flow and/or vascular resistance would be tenuous at best. However, since hypercapnia does produce marked changes on the blood flow and vascular resistance in various organs and tissues, an effect on the total blood content would not be unexpected. For if a tissue or organ is better perfused in blood flow concomitant with a vasodilation, the result would be an increase in total blood content of a sample of that

**The resistance to flow in a vascular bed is expressed as the ratio between arterial pressure and blood flow through the bed. One unit of peripheral resistance = 1 mm Hg/1 ml per mm Green et al. (1944)*

tissue or organ taken at a given time.

It has been known for a long time that an increase in carbon dioxide tension or a fall in pH causes an increase in relative red cell volume (Henderson, et al. 1924; Joffe and Poulton, 1922) and Jackson and Nutt (1954). These observations were based on in vitro experiments with the use of rather large CO_2 tensions, ranging up to 700 mm Hg in the case of Jackson and Nutt (1954). In vivo experiments of increased CO_2 on relative cell volume have not been reported, to our knowledge. Furthermore, the average CO_2 tension in our experiments was 123 mm Hg, a level at which CO_2 does not appear to influence significantly the relative red cell volume.

A detailed study on the effects of vasoconstrictor substances during respiratory acidosis in the cat has been done by Bygdeman (1963) which might have a bearing on the effects produced by hypercapnia. He demonstrated a decrease in the systemic blood pressure responses to injected noradrenaline correlated with the degree of acidosis and also a decrease in vascular reactivity to perfused noradrenaline in the unskinned leg, the kidney, and skinned leg. The vasoconstrictive effect of adrenaline and noradrenaline has been shown to be diminished during hypoventilation in normal cats, (Duzar and Fritz, 1959); and Duner and Euler, 1959) and in apneic dogs (Nahas, Ligou, and Mehlman, 1960).

MATERIALS

Male guinea pigs of the Hartley strain weighing between 350 and 600 grams were obtained from a commercial source. The animals were daily checked for any disease or deformities by a resident veterinarian and only those which showed an increase in body weight and no observable signs of infection were used in experiments.

Animals were exposed to 15% CO_2 in air (21% oxygen) for varying lengths of time in a plexiglass chamber rigged with food and water facilities. The gases used were obtained commercially and analyzed with a Scholander apparatus. The CO_2 concentration in the chamber was continuously monitored with a Beckman infrared CO_2 analyzer and the oxygen concentration intermittently with a Beckman O_2 analyzer. With these instruments the CO_2 concentration was held at $15 \pm 0.5\%$ and the oxygen at $21 \pm 1\%$. The chamber was installed in an air conditioned room which maintained the ambient chamber temperature at $24 \pm 2°$ C. Air within the chamber was continuously circulated through silica gel containers by means of a closed circuit system. Another closed circuit system, within the chamber, was equipped with boric acid containers to remove ammonia vapor. These systems maintained the environmental humidity at 65-75%. The exposure chamber was opened daily for a short time (2-3 minutes) for the removal of urine and feces and the replenishment of food and water.

The lack of any accessible superficial veins in the guinea pig necessitated a venial cut down for the injection of radio-biological materials. The vein chosen was the external jugular, which is easily exposed with only a small longitudinal incision approximately 2 centimeters (cm) from the midline at the base of the mandible beneath the foramen magnum. The animals on which total blood, plasma, and red cell volume determinations were done were not sutured because of the short time period before sampling. There was essentially no bleeding from incisions. All blood samples were taken via cardiac puncture.

All surgical procedures, including cardiac punctures, were carried out under pentobarbital anesthesia given interperitoneally at a dose level of 26 milligrams (mg) per Kg within 3-5 minutes. All of the animals recovered within 3-4 hours, apparently without ill effects at this dose level. Animals undergoing continuous exposure to carbon dioxide were only momentarily exposed to air while administering the anesthetic and briefly (30 seconds - 1 minute) for the cardiac puncture. Those animals on which the jugular vein was surgically exposed for injection of radio-biological materials were in an air environment for a somewhat longer period of time, approximately 1-3 minutes, and immediately returned to the chamber until the injected materials attained dilution equilibrium.

PART I: COMPARISON OF METHODS FOR MEASUREMENT OF TOTAL BLOOD VOLUME, RED CELL, AND PLASMA VOLUME

Total Blood Volume and Plasma Volume Determination by Albumen Tagged with I-131

Human serum albumin tagged with I-131 was obtained from Squibb Pharmaceuticals (containing 50-65 mg protein per ml with a specific activity of approximately 10μc per ml). The commercially obtained solution of tagged albumin was diluted with normal saline (0.9% NaCl) to a final concentration of 2-5 micro-curies per ml. For small animals, as used in these experiments, this range of specific activity is more than adequate to obtain high counts in small samples with good efficiencies (Everett, Simmonds and Lasher, 1956; Dewey and Hunter, 1960; Ormond and Rivera-Velez, 1965). The pH of the solution was adjusted to 7.41 with concentrated hydrochloric acid, which is the pH of guinea pig blood drawn from the abdominal aorta (Schaefer, McCabe, and Withers, 1968). A volume of 0.5 mls of the tagged albumin solution was injected into the surgically exposed jugular vein under light pentobarbital anesthesia, as described above. The injection procedure took approximately 30 seconds, using a plastic tuberculin syringe and a number 25 gauge needle. The syringe was fitted with a holder device to standardize the injected volume. The holder device facilitated the injection of the desired volume with less than one percent error. Reeve and

Frank (1956) reported the progressive absorption of radio-active iodinated albumin in dilute solution on glassware which was of a magnitude that could significantly alter the volume determinations. However, the use of the plastic tuberculin syringe avoids this possible technical error. Six washings of the syringe with dichromic acid solution always yielded less than 1 percent of the initial activity. To further reduce the possibility of error, the same syringe, with thorough washings in between, was used to determine the volume of standard I-131 albumin solution, injected solution and the one ml sample drawn via cardiac puncture. Triplicate samples drawn from the heart in this method showed good agreement with one another (+ 1 percent). Although no significant amount of I-131 albumin absorption could be detected, in the plastic syringe, care was taken to fill the syringe just prior to injection and to prepare the standard at the same time. A minimum time of 15 minutes was allowed for dilution (Fig. 1, Table I).

All whole blood samples and plasma samples were taken in duplicate and counted three times for a minimum of 10,000 counts on a Picker Spectrascaler II well-type gamma counter set at 0.354 MeV (window width 20). Samples were always counted in a final volume of one ml in disposable capped plastic tubes. The tagged human albumin prepared by Squibb Pharmaceuticals has approximately one atom of I-131 incorporated into the tyrosin moiety of each albumin molecule.

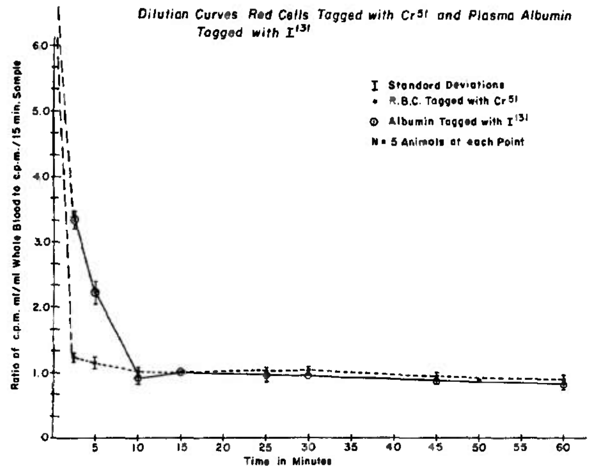

Fig. 1. Dilution curves red cells tagged with Cr^{51} and plasma albumin tagged with I^{131}

Calculations

The calculation of total blood volume and plasma volume are simple dilution problems (Gibson, et al., 1946):

(a) plasma volume (ml) =

$$\frac{\text{cpm injected per ml - background cpm}}{\text{plasma cpm per ml - background cpm}}$$

(b) total blood volume (mls) =

$$\frac{\text{cpm injected - background cpm}}{\text{cpm whole blood - background cpm}}$$

(c) Indirect determination of red cell volume according to Whipple, Hooper, and Robscheit (1920).

$$CV_h = PV \times \frac{H_c}{1 - H_c}$$

where CV_h = cell volume

PV = distribution of plasma label (nl)

H_c = the central hematocrit corrected for trapped plasma

TABLE I

DILUTION CURVES FOR Cr^{51} TAGGED RED BLOOD CELLS AND I^{131} TAGGED SERUM ALBUMIN IN GUINEA PIGS

(Expressed as the ratio of cpm ml^{-1} sample at "x" time to cpm ml^{-1} at 15 min. and Standard Error)

Time in minutes	Cr^{51} Tagged R.B.C.	I^{131} Tagged albumin*
0	10.15±2.14	11.09±3.50
2	1.22±0.17	3.34±2.16
5	1.13±0.07	2.21±1.68
10	1.03±0.05	0.97±0.28
15	(1)	(1)
20	---	0.99±0.08
30	1.05±0.03	0.98±0.04
45	0.99±0.08	0.89±0.06
60	0.96± .07	0.84± .07

* I^{125} Albumin gives comparable results. There are at least 5 animals at each time point. Expressed as the ratio to 15 min. (time used for sampling) to normalize differences in cpm injected, body weight, and blood volumes.

Determination of Total Blood, Red Cell and Plasma Volume by Cr-51 Tagged Red Blood Cells

The tagging of erythrocytes was accomplished with modification of the methods of Gray and Sterling (1950) and Cooper and Owen (1956). Blood was drawn from a guinea pig via cardiac puncture using Strumia Citrate-Dextrose solution as an anticoagulant. The Strumia or ACD solution as described by Strumia (1954) consists of 2.55 grams disodium citrate; 0.80 grams citric acid; 1.20 grams dextrose; and water to make 100 mls. The whole blood was immediately added to an equal volume of ACD solution. After gently mixing for a minute, an amount of $Na_2CR^{51}0_4$ was added to equal 20 microcuries per ml of whole blood. The mixture was incubated at room temperature with gentle mixing on a Yankee Variable Speed Rotator. After 45 minutes of incubation, ascorbic acid (1 mg per ml) was added and the mixture allowed to shake gently another five minutes. The ascorbic acid reduces the hexavalent chromium ion to its trivalent form. The mixture was centrifuged for 15 minutes at 3,000 r.p.m. and the plasma and ACD solution removed by careful aspiration. The tagged red cells were carefully washed three times with sterile saline. Table II shows a typical labeling experiment demonstrating the efficiency of labeling and of the wash procedure. The efficiency of labeling in six experiments was 91 $\pm$ 2.4% with a range of 85% to 95%. The last wash always contained less than one percent of the initial activity. The packed red cells were reconstituted to the original whole blood volume with sterile saline. One

TABLE II

EXAMPLE OF THE EFFICIENCY OF LABELING AND WASH PROCEDURE

Sample Source	Percent of Original Cr^{51}
Plasma	10.0*
1^{st} wash	1.6
2^{nd} wash	0.18
3^{rd} wash	0.09

*Range of labeling efficiency 70-95 percent.

ml of the reconstituted blood was injected and sampled as described for the I-131 tagged albumin. Duplicate whole blood samples were counted in a one ml volume three times for a minimum of 15 minutes or 10,000 counts on a Picker Spectrascaler II set at 0.320 MeV (window width of 70). Actual red cell determinations were made after carefully washing the cells three times with saline.

The range of chromium metal concentration used in these experiments ranged from 0.04 to 0.09 g (microgram) per ml blood. Since the guinea pigs are of a highly inbred strain, no immunological response was anticipated from the red cells obtained from one animal and injected into another and none could be detected by monitoring of the body temperature. The blood used was always fresh. The minimum

circulation time allowed was the same as the tagged albumin 15 minutes (Fig. 1, Table II).

Calculation of red cell volume, total blood volume and indirect estimation of plasma volume by CR-51:

(a) Total blood volume (ml) =

$$\frac{\text{cpm per ml of injected material}}{\text{cpm per ml of sample}}$$

(b) Red cell volume (ml) =

$$\frac{\text{cpm per ml of red cells injected}}{\text{cpm per ml of red cells in sample}}$$

(c) Plasma volume (ml) =

total blood volume x (1-microhematocrit)

Combined Method of Blood Volume Determination by Cr-51 Tagged Red Blood Cells and I-125 Tagged Albumin

The use of I-125 tagged human albumin (Squibb Pharmaceuticals) containing approximately 10 mg of protein and a specific activity of .05 mc per ml. with a gamma radiation energy at 35.4 kev. facilitates the simultaneous estimation of plasma volume and red cell volume tagged with Cr-51, since the Cr-51 has a gamma radiation energy of 0.270 MeV. (See Fig. 2).

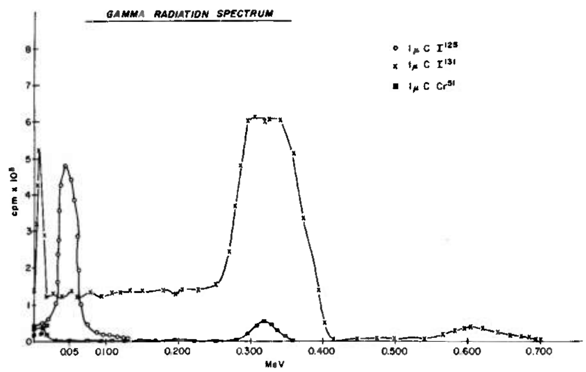

Fig. 2. *Gamma radiation spectrum*

The red blood cells were prepared as previously described and suspended in an equal volume of I-125 tagged albumin solution. One ml. of this suspension containing approximately 10-20 μc of CR-51 and 5 μc of I-125 was injected via the surgically exposed jugular vein as previously described. The dilution time was the same for tagged I-125 albumin as with I-131 tagged albumin. The two differently tagged human albumins also gave identical volume estimations (see Table III).

As demonstrated by Figures 3 and 4, there was essentially no interference from either Cr-51 in varying concentrations in the iodine-125 counting window (MeV. .050) or I-125 in varying concentrations in the chromium-51 counting window (MeV. 0.320-window width 70), throughout the activity ranges used in these experiments.

Iodine-125 emits X-rays and gamma rays with radiation energies of 27.5 kev. and 35.4 kev., respectively. The half-life iodine-125 is considered to be 57.4 days.

TABLE III

COMPARISON OF I-125 AND I-131 TAGGED ALBUMIN METHODS

Method	Total Blood Volume (mls Kg Body Weight)	S.E.	No. of Animals
I-125	86:84	$\pm$3:68	13
I-131	86.20	$\pm$4.98	23

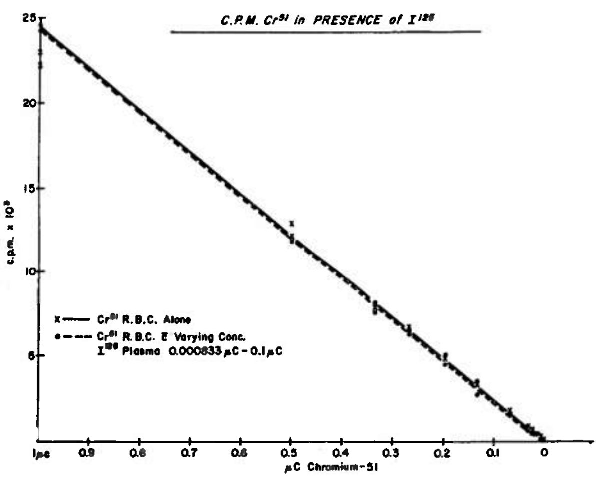

Fig. 3. C.P.M. Cr^{51} in presence of I^{125}

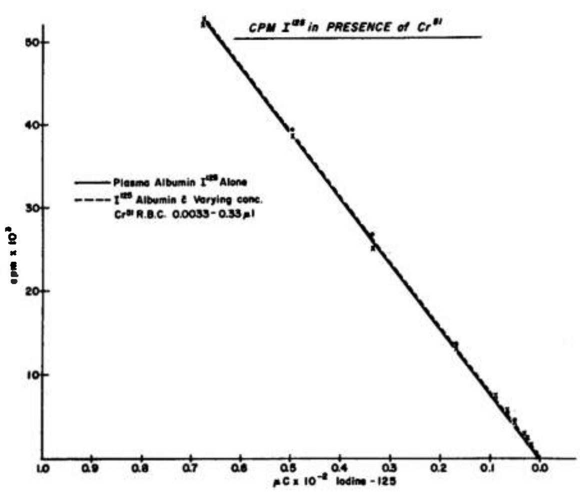

Fig. 4. C.P.M. I^{125} in presence of Cr^{51}

All samples were taken in duplicate and counted three times for a minimum of 10, 000 counts in a one ml. volume with a Picker Spectrascaler II.

Calculations of Total Blood Volume; Red Cell Volume; Plasma Volume; and Whole Body Hematocrit by Combined Use of Cr-51 and I-125

(a) Red Cell Volume (ml) =

$$\frac{\text{cpm RBC per ml. injected}}{\text{cpm RBC per ml. sample}}$$

(b) Plasma Volume (ml) =

$$\frac{\text{cpm per ml. plasma injected}}{\text{cpm per ml. plasma sample}}$$

(c) Total Blood Volume (ml) - Red cell volume + plasma volume

(d) Whole Body Hematocrit =

$$\frac{\text{Red cell volume}}{\text{Red cell + plasma volume}} \times 100$$

TRAPPED PLASMA DETERMINATIONS

Trapped plasma was determined from whole blood sample which had been labeled with I-131 tagged albumin. One ml samples were centrifuged at 10,000 r.p.m. for one hour. The plasma was carefully and scrupulously removed and assessed. This value, plus the values obtained from three washings of the packed red cell volume, was taken as 100 percent. That percentage of the assayed I-131 remaining in the red cell column was taken as trapped plasma (Klement, Ayen, and McIntyre, 1954).

Calculations:

$$\text{Trapped plasma} = 1 - \frac{\text{cpm per volume cells}}{\text{cpm per volume plasma}}$$

The amount of plasma trapped in the red cell column during centrifugation for one hour at 10,000 g was determined. The mean percent of plasma was 5.76 ± 1.10 in eleven one-ml. blood samples from normal guinea pigs. This value falls well within the range of trapped plasma determinations reported by other investigators: 2.0 to 8.5 percent (Chaplin and Ross, 1942; Mayerson, et al., 1948; Chaplin and Mollison, 1952; Hlad and Holmes, 1953; and Gregersen and Rawson, 1959).

MICRO-HEMATOCRIT DETERMINATIONS

Micro-hematocrits were determined in triplicate on each animal from blood drawn via cardiac puncture. The blood was drawn in heparinized capillary tubes (maximum taper 0.025 mm in 75 mm - A. H. Thomas Co.) and centrifuged for 10 minutes at 10,000 r.p.m. The readings were obtained with an International Micro-capillary Reader and did not include the buffy coat (mostly white blood cells). The micro-hematocrits were corrected for trapped plasma and for whole body hematocrit according to Chaplin, Mollison and Vetter, (1953) and Dewey, (1960) for use in the indirect estimation of plasma or red cell volume.

Calculation

Whole body hematocrit =

$$\text{micro-hematocrits} \times 0.96 \times 0.91$$

RESULTS OF METHODOLOGY

Table I and Figure 1 demonstrate that the dilution equilibrium time for red cells tagged with chromium-51 is essentially completed in two minutes or less and that there is no significant change between 10 and 60 minutes. The results of the time dilution curves are expressed as the ratio of the counts per minute (c.p.m.) per ml. of whole blood to the c.p.m. per ml. of the 15 minute sample, to normalize any differences in the c.p.m. injected, the body weights, and blood volumes between the different animals. These data are in agreement with the results of Pareira, Serkes, and Lang (1960). They found that the distribution space using chromium-51 tagged red cells was essentially unchanged at 15, 30, and 60 minutes in normal rats.

Figure 1 gives the time dilution curve for red cells tagged with chromium-51 in animals of approximately the same body weight and blood volume given the same specific activity of chromium-51.

The results of the iodinated albumin time concentration curve are also given in Table I and Figure 1. Approximately 10 minutes was required for iodinated albumin (I-125 and I-131) to reach dilution equilibrium. This time compares well with the average value of 9 minutes reported as the dilution equilibrium time of I-131 albumin in man (Pritchard, Moir, and MacIntyre, 1955).

The results of determination of total blood, red cell, and plasma volumes by the use of chromium-51 tagged red cells, iodinated human albumin (I-125) and by simultaneous estimation of red cell and plasma volume in the same untreated guinea pigs are presented in Table IV. The value obtained by the sum of the whole blood components, red cell volume, and plasma volume is referred to as the actual determination. This value more accurately reflects an estimation of blood volume than either method alone (Brown, Hopper and Wennesland, 1957; Gregersen and Rawson, 1959; and Mayerson, 1965). The estimation of total blood volume by

TABLE IV

TOTAL BLOOD VOLUME; RED CELL VOLUME; PLASMA VOLUME; AND HEMATOCRIT BY Cr^{51}; I^{125} AND COMBINED ACTUAL DETERMINATION BY Cr^{51} AND I^{125} IN THE SAME 13 UNTREATED GUINEA PIGS. MEAN VALUES EXPRESSED AS mls Kg BODY WEIGHT^{-1} ($\pm$ STANDARD ERROR).

METHOD	TOTAL BLOOD VOLUME	RED CELL VOLUME	PLASMA VOLUME	HEMATOCRIT
Cr^{51} + I^{125} (combined)	74.21$\pm$2.67	30.87$\pm$1.06	43.86$\pm$2.86	41.59$\pm$1.49
Cr^{51}	59.81$\pm$1.78	22.19$\pm$0.68	37.62$\pm$2.23	37.10$\pm$1.57
percent difference from actual	20↓	28↓	14↓	
I^{125}	86.83$\pm$3.60	32.40$\pm$1.27	54.43+1.80	37.31$\pm$1.82
percent difference from actual	17↑	5↑	19↑	

the chromium-51 method, which reflects the dilution of tagged red cells throughout the erythrocyte space within the circulatory system, gave a value of 59.81 ± 1.78 mls per Kg of body weight. This value was 20 percent lower than the value obtained by the actual determination, 74.21 ± 2.67 mls per Kg of body weight. Conversely, the estimation of total blood volume by I-125 tagged human albumin, which reflects the plasma circulatory space, over-estimated the blood volume by 17 percent in comparison to the actual determination. The percent differences from the actual determination of cell and plasma volume by either method alone demonstrated more erratic changes, which can in part be attributed to the numerous errors encountered in the use of peripheral micro-hematocrits from which they are determined.

DISCUSSION

Methods of Blood, Red Cell, and Plasma Determination

The methods of blood volume determination have long been a subject of controversy (Mayerson et al., 1948; Sjostrand, 1953; Brown, Hopper and Wennesland, 1957; Gregerson, 1959; and Mayerson, 1965). The reasons for the widespread and often heated controversy stem primarily from technical errors which are intrinsic to all methods and from misinterpretation of physiological phenomena.

The validity of any dilution method employed for the determination of blood volume can be assessed in relation to three general sources of error: (1) the accuracy with which the concentration of the test material can be determined in blood; (2) the recognition of the effects of uneven distribution of plasma and cells within the total circulation; and (3) the correct interpretations of the time concentration curve of the test material.

The use of isotopes with a high specific activity does give an accurate means of determining the concentration in the blood (Fine and Seligman, 1944; Peacock et al., 1946; and Gray and Sterling, 1950). While the determination of both blood compartments minimizes the error introduced by local variations in red cell and plasma distribution, which can be considerable (Allen and Reeve, 1953; Reeve et al., 1953, Everett, Simmons and Lasher, 1956; Friedman, 1959; Dewey, 1960; and Remington and Baker, 1961). Furthermore, the simultaneous determination of red cell and plasma volume, independently, avoids the use of the unreliable peripheral hematocrit, which is not comparable to the whole body hematocrit, for indirect determination of cell or blood volume (Malassez, 1874; Chaplin and Ross, 1942, Gibson et al., 1946; Chaplin and Mollison, 1952; Gray and Frank, 1953; Hlad and Holmes, 1953; and Berman, Carr and Malone, 1964).

A correct interpretation of the time concentration curve is dependent upon a knowledge of the behavior of test material *in vivo* and the physiological state of the organism. The biological half-life of labeled protein or cells, extravascular exchange or loss, rates of elution, degree of sequestration, anesthesia, physical well-being, are

but a few factors known to affect the time concentration curve (Berlin, Lawrence and Gartland, 1950; Read, 1954; Groom, Morris and Rowlands, 1957; Friedman, 1957; Dewey, 1959; and Dewey and Hunter, 1960).

The method described here employs the simultaneous use of chromium-51 for determination of red cell volume and of iodine-125 tagged human serum albumin for determination of the plasma volume. The total blood volume being the sum of these two independent measurements.

Chromium-51 and iodine-125 both emit gamma rays; however, their gamma-radiation energy peaks are at 0.270 and 0.0354 MeV. (million electron volts) respectively, and are effectively excluded from one another in counting.

Data obtained on blood volume, red cell volume and plasma volume of guinea pigs in these studies agree well with values reported in the literature, as shown in Table V.

NOTE: During the preparation of this manuscript, the work of Grable and Williams (1969) came to our attention. These authors reported the simultaneous use of CR-51 and I-125 for the determination of red cell and plasma volumes, respectively, in an experimental design similar to the one described here.

TABLE V

COMPARISON OF DATA OF BLOOD, RED CELL, AND PLASMA VOLUME REPORTED FOR GUINEA PIGS IN RECENT LITERATURE

Blood Volume ml Kg bd. wt^{-1}	Red Cell Volume ml Kg bd. wt^{-1}	Plasma Volume ml Kg bd. wt^{-1}	Method ml Kg bd. wt^{-1}	Reference
75.3±7.1 (13)	-----	39.1±3.7 (13)	I^{131}	Masouredis and Melcher (1951)
72.0±3.1 (18)	-----	38.6±2.8 (18)	Evans blue	Ancill (1955)
72-78	31-33	36-45	Evans blue	Constable (1963)
74.21±2.69 (13)	30.87±1.09 (13)	43.86±2.86 (13)	Cr^{51} & I^{125}	Baker (1969)

PART II: EFFECT OF CHRONIC HYPERCAPNIA ON TOTAL BLOOD VOLUME, RED CELL VOLUME, AND PLASMA VOLUME

RESULTS

Data on blood volumes obtained in two series of experiments with chronic hypercapnia using chromium-51, iodine-131 and iodine-125 are given in Tables VI and VII.

Blood volume determined by the chromium-51 method in 33 control animals of the first series was found to average 64.43 ml/kg body weight which is somewhat higher than values obtained with the same method in 13 animals during the second experiment (59.8 ml/kg); however, the differences are not statistically significant.

Blood volume measurements on control animals using iodine-131 in the first series and I-125 in the second series were in excellent agreement (Table VII).

Table VIII shows the results of the combined determination of red cell and plasma volume using chromium-51 and I-125 in the second series of experiments.

These data are used for the evaluation of hypercapnic effects on blood volume. There is a significant rise in total blood volume at one hour, three days and seven days of exposure to 15% CO_2 with a return to control levels after one day recovery on air following six days of exposure to CO_2.

Table IX demonstrates that the increased blood volume, seen during chronic hypercapnia, is due to the increase in red cell volume. There was no significant change in the plasma volume throughout the exposure period. A slight tendency for the plasma volume to increase above the control (8.7 percent) was observed after one hour. Red cell volume rose 13.6 percent after one hour of exposure to 15 percent carbon dioxide and at three days exposure showed an increase of 45.9 percent. In animals exposed for six days and then allowed a one-day recovery on air, the red cell volume dropped to slightly less than the control value.

In Table X whole body hematocrit values obtained by measurements of red cell volume and plasma volumes are compared with microhematocrit values. A significant increase in whole body hematocrit was found after three and seven days exposure to 15% CO_2 corresponding to the rise in red cell volume. Correlation factors for conversion of microhematocrit to whole body hematocrit are listed in Table XI. The effects of prolonged exposure of guinea pigs to 15% CO_2 on reticulocytes is shown in Table XII.

DISCUSSION

Effects of Chronic Hypercapnia on Blood Volume

To interpret the results of studies on the effect of chronic hypercapnia on blood volume, it is necessary to know the time course of the two important

TABLE VI

EFFECT OF CHRONIC HYPERCAPNIA ON TOTAL BLOOD VOLUME

COMPARISON OF TWO SERIES OF EXPERIMENTS

TOTAL BLOOD VOLUME (mls Kg BODY WEIGHT^{-1}) BY Cr^{51} TAGGED RED BLOOD CELLS

Method	Controls	1 Hour	1 Day	3 Day	7 Day	6 Day Exposure to CO_2 1 Day Recovery on Air
		15% CO_2				
First Series						
Mean±S.E.	64.43±2.71		69.43±2.71	68.72±2.58	72.90±2.26	
Number of Animals	(33)		(14)	(15)	(13)	
Probability compared to control	----		----	----	.01	
% change from control	----		7.76%	6.65%	13.14%	
Second Series						
Number of Animals	n=13	n=5	n=6	n=8	n=5	n=3
Mean	59.81	68.30	68.10	64.96	66.95	72.03
S.E.	±1.78	±2.47	±3.52	±3.78	±4.20	±2.38
P. Value	----	.02	.02	----	----	.01
% change	----	14.2%	13.9%	8.6%	11.9%	20.4%
Corresponding values in these two series of experiments are not statistically different from each other.						

TABLE VII

EFFECT OF CHRONIC HYPERCAPNIA ON TOTAL BLOOD VOLUME

COMPARISON OF TWO SERIES OF EXPERIMENTS

TOTAL BLOOD VOLUME (mls Kg BODY WEIGHT^{-1}) BY I^{125} AND I^{131} TAGGED ALBUMIN

Method I^{131}	Controls	1 Hour	1 Day	3 Day	7 Day
First Series					
$\pm$ S.E.	86.58$\pm$3.51		92.22$\pm$4.10	87.75$\pm$7.75	84.83$\pm$7.65
Number of Animals	(42)		(16)	(15)	(12)
Probability compared to control	---		.02	---	---
% change from control	---		6.98%	1.8%	1.6%
Second Series					
Number of Animals	13	5	6	8	5
Mean I^{125}	86.83	93.58	92.78	95.13	84.85
S.E.	$\pm$3.60	$\pm$2.72	$\pm$6.20	$\pm$6.58	$\pm$3.72
P. Value	---	---	---	.05	---
% change I^{125} & I^{131} tagged Albumin mean	---	7.8%	6.8%	9.6%	9.8%

Probability: Student's "T" test as compared to control, not given unless statistically significant at the 0.05 level. Corresponding values in these 2 series of experiments are not statistically different from each other.

TABLE VIII

EFFECT OF CHRONIC HYPERCAPNIA ON TOTAL BLOOD VOLUME OF GUINEA PIGS ESTIMATED BY SIMULTANEOUS DETERMINATIONS OF RED CELL AND PLASMA VOLUMES (ml Kg BODY WEIGHT^{-1})

Method	Control	1 Hour	1 Day	3 Day	7 Day	6 Day Exposure 1 Day Recovery on Air
Mean	74.21	82.04	79.52	89.06	80.47	74.10
S.E.	± 2.69	± 3.96	± 5.30	± 4.57	± 4.91	± 3.00
Probability Value	---	$< .001$	---	$< .001$	$< .05$	---
Percent change	---	10.5	7.2	20.0	8.4	---
Number of Animals	13	3	6	8	5	3

Probability: Student's "T" test as compared to Control, not given unless statistically significant at the 0.05 level.

TABLE IX

RED CELL AND PLASMA VOLUME DETERMINED BY Cr^{51} AND I^{125} RESPECTIVELY, IN GUINEA PIGS DURING CHRONIC HYPERCAPNIA

Methods	Control	1 Hour	1 Day	3 Day	7 Day	6 Day Exposure to CO_2 1 Day Recovery on Air
Number of Animals	(13)	(5)	(6)	(8)	(5)	(3)
Red Cell Volume mls Kg Body Weight^{-1}	30.87	35.08	33.12	45.05	37.45	30.60
Standard Error$\pm$	1.09	0.83	3.90	2.57	3.32	0.97
Probability compared to control	----	<0.05	----	<0.001	<0.05	----
% change from control	----	13.6% ↑	7.3% ↑	45.9% ↑	21.3% ↑	1.0% ↓
Plasma Volume mls Kg Body Weight^{-1}	43.86	47.70	46.12	44.02	43.02	43.30
Standard Error$\pm$	2.86	2.03	2.05	3.37	4.92	2.22
Probability compared to control	----	----	----	----	----	----
% change from control	----	8.7% ↑	5.1% ↑	0.4% ↑	1.9% ↓	1.3% ↓

Probability: Student's "T" test as compared to control, not given unless statistically significant at the 0.05 level.

TABLE X

HEMATOCRITS: WHOLE BODY BY ACTUAL DETERMINATION OF RED BLOOD CELL AND PLASMA VOLUME COMPARED TO MICROHEMATOCRITS IN THE SAME GUINEA PIGS DURING CHRONIC HYPERCAPNIA

Method	Control	1 Hour	1 Day	3 Day	7 Day	6 Day Exposure to CO_2 1 Day Recovery on Air
		← 15% CO_2 →				
Number of Animals	(13)	(5)	(6)	(8)	(5)	(3)
Actual	41.02	43.5	40.61	49.83	46.06	41.57
Standard Error ±	1.49	0.69	2.49	2.32	1.16	1.68
Probability compared to control	----	----	----	<.01	<.02	----
*Micro	42.89	42.8	37.5	46.31	41.85	42.26
Standard Error ±	1.66	0.88	0.79	0.99	1.83	1.33
Probability compared to control	----	----	----	<.02	----	----

*Microhematocrits - average of three determinations

Probability: Student's "T" test as compared to control, not given unless statistically significant at the 0.05 level.

TABLE XI

CORRECTION FACTORS FOR CONVERSION OF MICROHEMATOCRITS TO WHOLE BODY HEMATOCRITS DURING CHRONIC HYPERCAPNIA

	Control	1 Hour	1 Day	3 Day	7 Day	6 Day Recovery 1 Day Exposure on Air
		← 15% CO_2 →				
Correction factors	0.95	1.02	1.08	1.08	1.10	0.98

TABLE XII

EFFECT OF CHRONIC HYPERCAPNIA (15% CO_2 in 21% O_2) ON SIMULTANEOUSLY OBTAINED MEASUREMENTS OF MICROHEMATOCRITS AND RETICULOCYTES

		Hematocrit	Reticulocytes thousands per cu mm
Condition			
Control	Mean	40.6	17.1
	S.E.M.	.6	1.4
	N	10	10
15% CO_2			
1 Hour	Mean	39.0	22.2
	S.E.M.	1.4	2.7
	N	5	5
6 Hours	Mean	41.4	54.2*
	S.E.M.	1.1	2.7
	N	6	5
1 Day	Mean	41.8	34.3*
	S.E.M.	1.1	3.2
	N	6	6
3 Days	Mean	36.8	49.2*
	S.E.M.	1.8	5.8
	N	6	6
7 Days	Mean	35.2*	24.0
	S.E.M.	1.9	5.6
	N	6	6

*Differences from controls statistically significant at the 2% level and better.

parameters of acid base balance blood pH and P_{CO_2}, which have been shown to vary independently from each other in chronic respiratory acidosis and affect physiological functions in a specific way (Schaefer, 1961a, 1961b).

pH and P_{CO_2} of whole blood of guinea pigs exposed to the same conditions (15% CO_2 and 21% O_2) for prolonged periods of time was measured in separate investigations (Schaefer, Messier and Morgan, 1969). The results are used here in the discussion to delineate the unspecific pH effects from specific effects of increased CO_2 tension. The pH fell from a control level of 7.37 to 7.02 and 7.01 at one hour and one day respectively. From then on, compensatory mechanisms began to take effect, the pH rose to 7.11 at three days and 7.225 at seven days.

The time course of the pH changes during prolonged exposure to 15 percent CO_2 clearly displays the two phases of uncompensated and compensated respiratory acidosis. The exact time at which compensation is completed is difficult to determine because the pH is still rising at seven days. However, for practical purposes, the respiratory acidosis induced by exposure to 15 percent CO_2 has been considered to be compensated after three days, since at that time the organ changes (lung functions) and stress response produced by the respiratory acidosis have subsided (Schaefer, Avery and Bensch, 1964; Schaefer, McCabe and Withers, 1968).

In contrast to the biphasic changes of the pH, the P_{CO_2} remains at the elevated level of about 123 mm Hg attained during the first hour of exposure.

The increase in blood volume observed throughout the exposure to 15 percent CO_2 was statistically significant with the exception of the data obtained at one day, where the rise in blood volume was less than at one hour. The same changes were noted in the red cell volume while plasma volume did show a tendency to rise without reaching significant proportions. At one day recovery on air following six days exposure to 15 percent CO_2, blood volume, red cell volume and plasma volume returned promptly to normal levels.

The slight decrease in blood volume and red cell volume observed at one day separated a smaller peak at one hour from the much higher peak at three days following which there is another decline (Tables VIII and IX).

The stress response to 15 percent CO_2, consisting of epinephrine discharge and associated spleen contraction, was found to be limited to the uncompensated phase of respiratory acidosis and subsided by three days (Schaefer, McCabe and Withers, 1968). Since the spleen is known to function as a blood store which can accommodate, according to Barcroft (1934) in many animals about 16 percent of the blood volume, which is released in response to accumulation of CO_2 or epinephrine discharge, the observed increase of 10.5 percent in blood volume and 13.6 percent in red cell volume after one hour of exposure, could be accounted for by the release of blood stores. However, the blood volume increase of 20 percent and red

cell volume rise of 46 percent after three days exposure to 15 percent CO_2 are larger than the capacity of the spleen blood store, and furthermore, the spleen has returned at this time to the control levels of weight indicating that the contraction has subsided (Schaefer, McCabe and Withers, 1968). The increase in blood volume demonstrated at the third and seventh day of exposure must, therefore, have a different cause. An increasing effect of elevated P_{CO_2} on the relative cell volume has been widely demonstrated in vitro; however, assuming the P_{CO_2} increase seen here does have an effect, it would be less than one percent as calculated from the data of Jackson and Nutt (1954). Furthermore, the error would be introduced only in those calculations envolving the microhematocrit.

The degree of the augmentation of red cell volume after three days suggests the operation of an erythropoietic stimulation. This hypothesis is supported by findings showing a marked increase in reticulocytes of guinea pigs exposed to 15% CO_2 (Table XII).

Another factor which could contribute to a stimulation of erythropoiesis in hypercapnia is the increased hemolysis, which was regularly observed during exposure of guinea pigs to 15% CO_2.

Hypercapnia, induced by breathing 6.8 percent carbon dioxide in air, increases the formation of carbon monoxide (Malstrom and Sjostrand, 1953). This report is in agreement with in vitro studies where carbon monoxide formation increased when the pH of blood was shifted in the acid direction (Sjostrand, 1953). There appears to be a parallelism between the formation of carbon monoxide and the intravital breakdown of hemoglobin. The fact that hemoglobin is to some extent broken down in the circulating blood, necessitates the presence of circulating stroma or ghosts (hemolyzed erythrocytes minus the hemoglobin). Blood autohemolysates injected into anemic dogs accelerated their recovery, increasing the circulating hemoglobin by 60.7 percent (De La Cabadz, Labardini, and Sanchez-Medal, 1963). The concept of a feedback control of erythropoiesis by the breakdown products of red blood cells is not new. It has been shown that the heme fraction elicited an erythropoiëtic response when injected into fasted, dehydrated rats (Brown Altschuler, and Cooper (1963). Recently, it has been demonstrated that the stroma of erythrocytes and reticulocytes reinjected into rabbits enhanced the incorporation of methionine S-35 into the proteins by more than 50 percent. The stroma of erythrocytes and reticulocytes also enhanced the incorporation of P-32 into the nuclear fraction of bone marrow ribonucleic acid (RNA) by 77 percent and 134 percent, respectively. Furthermore, the erythrocyte stroma increased the synthesis of ribosomal (RNA) by 500 percent (Bezrukov, 1968).

Available evidence of a marked increase in reticulocytes and observations of an increased CO_2 induced hemolysis support the notion of an erythropoietic stimulation in chronic hypercapnia. However, it is not possible to distinguish between a direct effect of hypercapnia on increased red cell production or an indirect effect

through the accelerated destruction of erythrocytes, with their catabolic products being the erythropoietic stimulant.

It can be concluded that the smaller increase in red cell volume observed during the uncompensated phase of respiratory acidosis is related to the pH dependent epinephrine discharge which causes together with the increased CO_2 level in the blood a discharge of the blood stores. The larger subsequent increase in red cell volume must be related to an erythropoietic stimulation.

The changes in total blood volume and red cell volume observed under chronic hypercapnia may be predominantly related to pH effects although it is not possible to clearly separate the effects of increased CO_2 tension.

Peripheral versus Whole Body Hematocrit

The discrepancy between peripheral and whole body hematocrit is a well established fact (Gibson et al., 1946; Reeve et al., 1953; Rapaport et al., 1956; and Gregersen and Rawson, 1959). A constant relationship between peripherial and whole body hematocrit has been shown to exist and correction factors have been calculated to convert peripheral to whole body hematocrit by a number of investigators (Chaplin, Mollison and Vetter, 1953; Gregersen et al., 1959; and Lawson, 1962). The correction factor has been reported to range between 0.89 and 0.94, in man, between 0.917 and 1.0 in dogs, between 0.739 and 0.986 in rats, and between 0.73 and 0.88 in mice (Lawson, 1962). To our knowledge, no correction factor has been reported for guinea pigs. The factor calculated for normal guinea pigs of 0.95 is well within the range reported for the rat. However, the use of such a standard correction factor during altered physiological conditions, as noted here during chronic hypercapnia, is erroneous. If the factor calculated for the normal animal was used to convert peripheral to whole body hematocrit during chronic hypercapnia, the value would be in error by 15 percent at seven days exposure, since the ratio of peripheral to whole body hematocrit was actually higher (a correction factor of 1.10).

REFERENCES

Allen, T. H. and E. B. Reeve (1952). Am. J. Physiol. 175:218.

Ancill, R. J. (1956). J. Physiol. 132:469.

Baker, G. T. (1969). In press.

Barcroft, J. (1934). Cambridge Comparative Physiology, Cambridge University Press, Cambridge 335.

Berk, L. (1945). SouthAfrican Journal of Med. Sci. 10:95.

Berlin, N. J., J. H. Lawrence and J. Gartland (1950). Am. J. Med. 9:747.

Berman, I., R. Carr and E. Malone (1964). Nature. 202:1013.

Berzrukov, N. W. (1968). Patol. Fiziol. Eskp. Terap. 12:23.

Brackett, M. D., Jr., J. J. Cohen and W. B. Schwartz (1965). New England J. of Med. 272:6.

Brickner, E. W., E. G. Dowds, B. Willits and E. E. Selkurt (1956). Am. J. Physiol. 184:275.

Bronk, D. W. and R. Gesell (1927). Am. J. Physiol. 82:170.

Brown, J. R., N. Altschuler and J. A. D. Cooper (1963). Erythropoietic Effect of Red Blood Cell Components and Related Compound, Proc. Soc. Exptl. Biol. & Med. 112:840-843.

Brown, E., J. Hopper, Jr. and R. Wennesland (1957). Ann. Review of Physiol. 19:231.

Bygdemen, S. (1963). Acta Physiol. Scandinav. 61: Supp. 222.

Chaplin, M. A., and J. F. Ross (1942). Am. J. Physiol. 138:447.

Chaplin, H. and P. L. Mollison (1952). Blood. 7:1227.

Chaplin, H., P. L. Mollison and H. Vetter (1953). J. Clin. Invest. 32:1309.

Constable, B. J. (1963). J. Physiol. 167:228.

Cooper, M. and C. A. Owen (1956). J. Lab. and Clin. Med. 47:65.

Cooper, K. E. (1962). Ann. Review of Physiol. 24:139.

Crane, M. G. and R. Adams (1954). Am. J. Med. 17:118.

Dagher, F. J., A. Panossian and J. D. Fuleihan (1968). Am. J. Surgery 115:594.

De La Cabada, L. A., J. Labardini and L. Sanchez-Medel (1969). Proc. Soc. Exptl. Biol. and Med. 131:279.

Dewey, W. C. (1959). Am. J. Physiol. 197:423.

Dewey, W. C. (1960). Am. J. Physiol. 198:1011.

Dewey, W. C. and J. D. Hunter (1960). J. Appl. Physiol. 15:961.

Doisy, E. A. and E. P. Eaton (1921). J. Biol. Chem. 47:377.

Doisy, E. A. and J. W. Beckmann (1922). J. Biol. Chem. 54:683.

Dreyer, G. and W. Ray (1910). Phil. Trans. B 201:133.

Duner, H. and U.S.V. Euler (1959). Acta Physiol. Scand. 46:201.

Duzar, J. and G. Fritz (1924). Klin. Wschr. 3:2338.

Ebaugh, F. G., Jr., C. P. Emerson and J. F. Ross. J. Clin. Invest. 32:1260.

Erlanger, J. (1921). Physiological Review 1: 177.

Everett, N. B., B. Simmons and E. P. Lasher (1956). Circ. Res. IV:419.

Fine, J. and A. M. Seligman (1944). J. Clin. Invest. 23:720.

Fortier, C. (1949). Proc. Soc. Biol. and Med. 70:76.

Friedman, J. J. (1957). Am. J. Physiol. 189:246.

Friedman, J. J. (1959). Am. J. Physiol. 196:420.

Gibson, J. G., II, W. C. Peacock, A. M. Seligman and T. Sack. (1946). J. Clin. Invest. 25:838.

Gibson, J. G., II., A. M. Seligman, W. C. Peacock, J. C. Aub, J. Fine and R. D. Evans (1946). J. Clin. Invest. 25:848.

Giebisch, G., L. Berger and R. F. Pitts (1955). J. Clin. Invest. 34:1671.

Grable, E. and J. A. Williams (1969). J. Nuclear Med. 9:219.

Gray, S. J. and K. Sterling (1950). J. Clin. Invest. 29:1604.

Gray, S. J. and H. Frank (1953). J. Clin. Invest. 29:1000.

Gregersen, M. I. and R. A. Rawson (1959). Physiol. Review. 39:307.

Groom, A. C., W. B. Morris and S. Rowlands (1957). J. Physiol. 136:218.

Gscheidlen, R. (1869). Untersuch. Physiol. Lab. Wurzburg 3:141.

Henderson, L. J., A. V. Bock, H. Field and J. L. Stoddard (1924). J. Biol. Chem. 59:379.

Hilton, R. and F. Eicholtz (1925). J. Physiol. 59:413.

Hlad, C. J., Jr. and J. H. Holmes (1953). J. Appl. Physiol. 5:457.

Jackson, D. M. and M. E. Nutt (1954). J. Physiol. 123:367.

Joffe, J. and E. P. Poulton (1920). J. Physiol. 54:129.

King, C. T. G., E. E. Williams, J. L. Mego and K. E. Schaefer. (1955). Am. J. Physiol. 183:46.

Klement, A. W., Jr., D. E. Ayer and D. R. McIntyre (1954). Proc. Soc. Exptl. Biol. and Med. 87:81.

Lawson, H. (1962). Handbook of Physiology I. Circulation. Ed. Philip Dow, Waverly Press, pp. 7-23.

Lennox, W. G. and E. L. Gibbs (1932). J. Clin. Invest. 11:1155.

Jones, H. C. H. and P. L. Mollison (1956). Clin. Sci. 15:207.

Logsdon, D. F. and J. F. Green (1968). Report SAM-TR-68-69 USAF School of Aerospace Medicine, Brooks Air Force Base.

Lotspeich, W. D. (1967). Science. 155:1066.

Malassez, L. (1874). Arch. Physiol. Norm. Path. (second series) 1:797.

Malmstrom, G. and T. Sjostrand (1953). Acta Physiol. Scandinav. 27:231.

Masouredis, S. P. and L. R. Melcher (1951). Proc. Soc. Exptl. Biol. 78:264.

Mayerson, H. S., C. Lyons, W. Parson, R. T. Nieset, and M. V. Trautman (1948). Am. J. Physiol. 155:232.

Mayerson, H. S. (1965). Ann. Review of Physiol. 27:307.

Mellanby, J. and C. C. Wood (1922). J. Physiol. 57:113.

Nahas, G. C., J. C. Ligou and B. Mehlman (1960). Am. J. Physiol. 198:60.

Necheles, T. F., I. M. Weinstein and G. U. LeRoy (1953). J. Lab. and Clin. Med. 42:358.

Niemoeller, H. and K. E. Schaefer (1962). Proc. Soc. Exptl. Bicl. and Med. 110:804.

Ormond, A. P., Jr. and J. M. Rivera-Velez (1965). Proc. Soc. Exptl. Biol. and Med. 118:600.

Pareira, M. D., K. D. Serkes and S. Lang (1960). Proc. Soc. Exptl. Biol. and Med. 103:9.

Peacock, W. C., R. D. Evans, J. W. Iluime, Jr., W. M. Good, A. F. Kipp, S. Weiss and J. G. Gibson, II (1946). J. Clin. Invest. 25:605.

Pritchard, W. H., T. W. Moir and W. J. MacIntyre (1955). Circ. Res. 3:19.

Ranke, J. (1871). Die Blutvertheilung Und Der Tatigkeitswechsel Der Organe. Ed. Wilhelm Engelmann, Leipzig, p. 42.

Rapaport, E., H. Kuida, F. Haynes and L. Dexter (1956). Am. J. Physiol. 185:127.

Read, R. C. (1954). N. E. Journal of Medicine 250:1021.

Reeve, E. B., M. I. Gregersen, T. H. Allen, H. Sear and W. W. Walcott (1953). Am. J. Physiol. 175:204.

Reeve, E. B. and J. J. Franks (1956). Proc. Soc. Exptl. Biol. and Med. 93:299.

Reeve, E. B., T. H. Allen and J. E. Roberts (1960). Ann. Review Physiol. 22:349.

Reeve, E. B., M. I. Gregersen, T. H. Allen and H. Sear (1953). Am. J. Physiol. 175:195.

Remington, J. W. and C. H. Baker (1961). Circ. Res. IX:60.

Richards, J. B. (1957). Am. J. Physiol. 188:7.

Richardson, D. W., A. J. Wasserman and J. S. Patterson, Jr. (1961). J. Clin. Invest. 40:31.

Schaefer, K. E. (1964). Arch. Environ. Health. 9:320.

Schaefer, K. E., M. E. Avery and K. Bensch (1964). J. Clin. Invest. 43:2080.

Schaefer, K. E., M. Hasson and H. Niemoeller (1961a). Proc. Soc. Exptl. Biol. and Med. 107:355.

Schaefer, K. E. (1961b). Ann. N. Y. Acad. Sci. V92, Art 2, pp. 401-413.

Schaefer, K. E., B. J. Hastings, C. R. Carey and G. Nichols (1963). J. Appl. Physiol. 18:1071.

Schaefer, K. E., E. M. Jordan and A. J. Thelin. (1965). Abstract. Fed. Proc. 24:397.

Schaefer, K. E., H. Klein and K. A. Zink (1950). Klin. Wochschr. 28:179.

Schaefer, K. E., N. McCabe and J. Withers (1968). Am. J. Physiol. 213:543.

Schaefer, K. E., G. F. Bond, W. F. Mazzone, C. R. Carey and J. H. Dougherty, Jr. (1968). Aerospace Med. 39:1206.

Schmidt, D. F. (1928). Am. J. Physiol. 82:202.

Sjostrand, T. (1953). Physiol. Review. 33:202.

Smirk, F. H. (1928). Brit. J. Exptl. Pathology. 9:81

Smith, L. H. and T. W. McKinley (1962). Proc. Soc. Exptl. Biol. and Med. 111:768.

Steck, I. E. and E. Gellhorn (1939). Am. Heart J. 18:206.

Steinberg, J. (1873). Pflug. Arch. ges Physiol. 7:101.

Strumia, M. M. (1954). Am. J. Clin. Path. 24:260.

Strumia, M. M., L. Taylor, A. B. Sample, L. S. Colwell and A. Dugan (1955). Blood. 10:429.

Van Liew, H. D. (1963). Aerospace Medicine. 34:499.

Warburg, E. J. (1922). Biochemistry J. 16:153.

Weinstein, I. M. and G. U. LeRoy (1953). J. Lab. and Clin. Med. 82:368.

Went, S. and C. K. Drinker (1929). Am. J. Physiol. 88:468.

Yoffey, J. M. (1954). British Med. J. 11:193.

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)* NAVAL SUBMARINE MEDICAL CENTER, Submarine Medical Research Laboratory	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b. GROUP

3. REPORT TITLE

EFFECTS OF CHRONIC HYPERCAPNIA ON BLOOD VOLUME, PLASMA VOLUME AND RED CELL VOLUME IN RELATION TO THE SUBMARINE ENVIRONMENT

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

Interim Report

5. AUTHOR(S) *(First name, middle initial, last name)*

George T. Baker, III and Karl E. Schaefer

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
24 November 1969	26	104

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S) SMRL Report No. 604
b. PROJECT NO. MF12.524.006-9028	
c.	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10. DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY Naval Submarine Medical Center Box 600 Naval Submarine Base New London Groton, Connecticut 06340

13. ABSTRACT

Blood volume in guinea pigs, estimated on the basis of simultaneous measurement of red cell volume using chromium-51 tagged red cells and plasma volume with iodinated human albumin (I-125), was found to be more accurate than blood volume data calculated from separate determinations of red cell volume (chromium-51) and plasma volume (I-131). In chronic hypercapnia total blood and cell volume was found to increase during the uncompensated phase and increase to a greater extent during the compensated phase, while the plasma volume did not change significantly. The first rise was attributed to the release of blood stores from the spleen and liver while the later rise was attributed to an increased rate of erythropoiesis.

DD FORM 1 NOV 65 1473 (PAGE 1)

S/N 0101-807-6801

UNCLASSIFIED
Security Classification

3ND PPSO 13152

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Chronic hypercapnia						
Total blood volume						
Plasma volume						
Cell volume						
Erythropoiesis						